TUBERCULOSIS OF FISH

A Review of the Literature with a Description of the Disease in Salmonoid Fish

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The causes for the decrease in the catch of Pacific salmon over the past few years have been the subject of much controversy. A disease caused by an acid-fast organism and commonly called "fish tuberculosis," one of a number of possible serious diseases affecting adult fish, may be contributing to this loss to a much greater extent than has been previously realized.

The purpose of this review of the literature is to serve as an introductory and orienting report by bringing up to date the information on identification of the etiological agent, host range, and experimental work on pathogenicity.

Of the four species of *Mycobacterium* characterized in this paper, only two, *M. marinum* and *M. platypoecilis*, have been accepted by the 7th edition of *Bergey's Manual of Determinative Bacteriology* (Breed *et al.*, 1958). One species, *M. piscium*, has been dropped from the classification and the final one, *M. anabanti*, has never been accepted.

Bataillon *et al.* (1897) were the first to record the disease in fish when they reported its occurrence in a carp (*Cyprinus carpio*) in waters contaminated by sputa and excreta of a tuberculous person. A small, gray lesion was observed between the abdominal wall and the ovary. Excised, the lesion was histologically a tubercle and contained acid-fast bacilli. The organism grew well on synthetic media at 23 to 25 C and a rapid, flocculent growth was obtained in broth within 3 to 4 days. It was extremely pathogenic for frogs, in which it produced a disseminated type of disease. However, when reinoculated into carp, the original host, the fish showed resistance to the organism. It was pathogenic for lizards but not for pigeons or guinea pigs.

These authors conducted experiments to determine the origin of the bacillus and claimed to have adapted human tubercle bacillus to the fish. They felt that the reverse adaptation could occur and that a warm-blooded animal could be infected by the cold-blooded animal organism, although two of the authors were unsuccessful (Bataillon and Terre, 1897; Terre, 1902). Dubard (1897, 1898) adapted the acid-fast bacillus of carp to guinea pigs by serial passage of material in the warm-blooded animal.

The organism described by these three authors was named *Mycobacterium piscium* on the basis of its morphology, staining characteristics, and derivation (Bataillon *et al.*, 1902).

Auché and Hobbe (1897) refuted the claim of Bataillon *et al.* concerning the adaptation of the bacillus and presented observations that dead bacilli can produce lesions similar to those caused by live organisms in the frog. Many other authors including Nicolas and Lesieur (1899), Sion (1900), Lubarsch (1900), and Herr (1901)[1] supported arguments against this adaptation.

There are many investigators who support in part or entirely the theory that the human tubercle bacillus can be adapted to cold-blooded animals or the cold-blooded form to warm-blooded hosts. Moeller (1898) injected sputum of a tuberculous patient into a blind worm and a year later obtained a culture of the acid-fast bacilli that was nonpathogenic and nontransferable to rabbits. Dieudonné (1902, 1903) injected human tubercle bacilli into frogs. The animals lived but the bacilli were found to be alive 60 days after injection. Passage of the organism increased its virulence and it showed many of the characteristics of *M. piscium*. He was unable to restore the virulence for warm-blooded animals. Herzog (1902, 1903) studied the effects of passage of the acid-fast organism in cold-blooded animals and reported that the virulence for the cold-blooded animal is increased as the virulence for the warm-blooded animal is decreased or lost. Horman and Morgenroth

[1] The author wishes to express his appreciation to Mr. Gerald Oppenheimer and Mrs. Delores J. Parisot for their translations of the German publications.

(1899) fed sputa to carp and goldfish. No tuberculosis was found in the fish but tissue from these fish produced the disease in guinea pigs when the material was injected into these warm-blooded animals several months later. Bertarelli (1905) produced tuberculosis in the varanus (*Varanua varanus*) by injecting them with the sputum of a tuberculous patient. Aujeszky (1906) was able to adapt fish acid-fast bacilli to body temperature of warm-blooded animals after much difficulty. This adapted organism was pathogenic for smaller laboratory animals, killing guinea pigs in 36 to 68 days. He increased its virulence by serial passage so that it killed the test animal in 3 weeks. The organism was also pathogenic for rabbits. Calmette (1924) reviewed the studies of experimental infection of fish with mammalian tuberculosis.

Von Betegh (1910a, b) reported on experimental work with *M. piscium* in marine fishes and stated that the organism was nonpathogenic for them, although it was pathogenic for an eel (*Anguilla vulgaris*), a vertebrate, the female of which spends part of its life cycle in fresh water after spawning in sea water. The disease in this eel was localized and since the organism could not be recultured from the lesion, it was considered nonviable. He felt that the organism was pathogenic for fresh-water fish only and that because of its catadromous cycle, the eel represented an intermediate cycle host.

Bertarelli and Bocchia (1910) surveyed fish brought into several of the larger fish markets in Italy to determine the extent of the disease in natural populations. They did not find the organism in any of the fish they examined and concluded that the infection was extremely rare in high seas fish or not present at all.

It was not until 1913 that a spontaneous infection with acid-fast organisms was recorded in marine fish. At that time, Alexander (1913) and Johnstone (1913) reported their observations of the disease in the same fish, a cod (*Gadus callaria*). Johnstone's paper described the pathology of the infection and stated that formalin-preserved sections showed typical, tuberculous lesions. The lesions contained numerous bacilli which took the characteristic acid-fast strain.

The organism described by Alexander was obtained from a dark-colored area of the skin. He was unable to culture it except in the presence of cocci, and therefore could not characterize the organism. He injected suspensions of organisms and inserted pieces of the fish into incisions in guinea pigs to check the pathogenicity of the organism for this animal. One of the incised guinea pigs developed a lesion that became quite swollen and finally ruptured. Acid-fast bacilli were recovered from this lesion but not from the organs when the animal was autopsied. A second incised animal developed a lesion that subsided. None of the other test guinea pigs developed any signs of the disease. In this paper, Alexander also briefly reviewed the literature on fish tuberculosis.

Sutherland (1922) reported the bacilli in a halibut (*Hippoglossus hippoglossus*), where they were scattered throughout the viscera. The organism grew well at 20 C, but not at 37 C. The cultures were eventually lost due to contamination. Pathogenicity tests indicated it was not pathogenic for guinea pigs or frogs.

Aronson (1926) reported an acid-fast organism in the viscera of fish dying in the Philadelphia Zoo. He was able to culture the bacilli from a sergeant major (*Abedufduf mauritii*), three croakers (*Micropogon nalatus*), and two black sea bass (*Centropristes striatus*). The organism grew on Dorset's and Petroff's media at 18 to 22 C. No growth was obtained on glycerolated agar as a primary isolate, but later subcultures grew well on this medium. It would not grow at 37 C. On solid media it appeared as a gray-white, moist, elevated, irregular colony resembling avian tubercle bacillus type b and eventually developed a lemon yellow to deep orange color upon aging. It required from 14 to 18 days for initial growth, but grew more rapidly on subculture. In broth, growth was diffuse without pellicle formation. Morphologically, it was a pleomorphic, acid-fast, beaded or barred, long, thin, nonmotile, nonsporulated, gram-positive, nonbranching rod. The organism was not pathogenic for guinea pigs but very pathogenic for frogs. Aronson named it *Mycobacterium marinum* on the basis of isolation of the organism from marine fish, its morphology, pathogenicity, and cultural characteristics (table 1).

Johnstone (1927) received the posterior section of a condemned halibut sent to his laboratory with a request to identify the cause of its diseased conditions. The kidney was markedly swollen and covered with granulomatous tubercles that were pale yellow in color. The ovary

TABLE 1

Characteristics of acid-fast bacilli isolated from fish

	*Mycobacterium piscium**	*Mycobacterium marinum*†	*Mycobacterium platypoecilis*†	*Mycobacterium anabantii*‡
Reference	Bataillon *et al.* (1897)	Aronson (1926)	Baker and Hagan (1942)	Besse (1949)
Morphology	Acid - fast, gram-positive, non-motile, slender rods occasionally in threads.	Acid - fast, gram-positive, non-motile, pleomorphic, banded or beaded rods.	Acid - fast, gram-positive, straight rod; not as pleomorphic as the human bacillus.	Acid - fast, gram-positive, non-motile, nonsporulated, granulated rod.
Cultural characteristics:				
(a) Media	Glycerol agar, Dorset's medium, Heart Infusion Broth.	Glycerol agar, Heart Infusion Broth, Petroff's medium.	Glycerolated egg, glycerine phosphate, Sohngen's medium.	Loewenstein, Loeffler, Petragnani, Laporte, glycerolated potato.
(b) Temperature	23–25 C. Claimed to have adapted it to 37 C. Killed at 60 C for 1 hr.	18–20 C. No growth at 47 C. Killed at 60 C for one hour.	25 C. No growth at 37 C. Temperature range not investigated thoroughly.	25 C. 37 C is lethal. Range of growth 12–33 C.
(c) Growth time	1 week on agar, 3–4 days in broth.	14–18 days on agar; grew more rapidly when subcultured.	3 weeks on glycerolated agar.	15 days to 3 weeks.
(d) Colony characteristics	Flat, smooth, glistening, yellow on glycerol agar, green on Dorset's.	Smooth, moist, elevated, lemon-yellow to deep orange in old cultures.	Smooth and moist when young. Rough and dried when old. Deep orange color when cultured in light.	Appearance of cauliflower on potato medium. Cream to yellow orange color.
Pathogenicity	Frog, carp, eel, lizards. Not pigeons or guinea pigs.	Frogs, mice, pigeons, salt-water fish. Not rabbits or guinea pigs.	Mexican platyfish. Not for goldfish. No other animals tested.	Siamese fighting fish. Rainbow perch.
Source	Carp, fresh-water fish.	Sea bass, croaker, Sergeant major, salt-water fish.	Mexican platyfish, warm-water fish.	Siamese fighting fish, brackish-water fish.

* Not recognized by *Bergey's Manual* (7th Edition).
† Recognized by *Bergey's Manual* (7th Edition).
‡ Never recognized by *Bergey's Manual.*

was small and unripe in that no regular ovarian structure was seen in the sections. Stained sections showed characteristic granulomatous tuberculosis; however, giant cells were not present and the centers were caseous with a small number of acid-fast organisms present.

Griffith (1928) recorded the disease in captive wild animals and identified *M. marinum* in four caymans, brackish-water inhabitants, and *M. piscium* in one snake, a fresh-water inhabitant. This same author also observed acid-fast bacilli in the roe of a halibut but did not identify them.

Baker and Hagan (1942) recorded the disease in the Mexican platyfish (*Platypoecilis maculatus*), sixteen of which were brought to their laboratory. The fish were dull in color, sluggish, emaciated, and several were dead on arrival. There was an ulcer in the dorsal fin area of one and around the mouth of another. Smears from the ulcer, liver, spleen, and gills of the fish with the ulcerated fin contained acid-fast organisms. Cultures from liver, spleen, and kidney of eleven of the remaining fish were obtained on glycerolated egg at 25 C. No growth occurred on glycerolated phosphate or Sohngen's medium or at 37 C on any medium. Three weeks were required for the initial microscopic growth.

Colonies were smooth and moist when young but became rough and dry as they aged. They developed a deep orange color when cultivated in the light but remained cream-colored in the dark. The organism was an acid-fast, straight rod that did not display as much pleomorphism as human forms of the tubercle bacillus. It was nonpathogenic for goldfish; no other animals were tested. Baker and Hagan felt that on the basis of source, morphology, and comparison to their extensive acid-fast collection, the organism they described was sufficiently different to be named *M. platypoecilis*.

In 1949, the French worker Pierre Besse described what he considered to be a new species of *Mycobacterium* and named it *M. anabanti* after the family name of the group of fish from which he isolated the organism. This group, Siamese fighting fish (*Macropodus opercularis*), died in their tank in the Museum of France, Over-seas. An organism was isolated by Besse which he characterized as follows:

A variety of media, both solid and liquid, were tested and growth was obtained on Loewenstein, Laporte, Petragnani, Loeffler, Jensen, Jensen glycerine, potato with glycerine, and coagulated serum media. Growth was slow, requiring from 15 days to 3 weeks for the first appearance of minute colonies. Temperatures for growth ranged from 12 to 33 C with the optimum at 25 C. The author found 37 C to be lethal to cultures exposed to that temperature for 48 hr. Color of colonies ranged from cream to yellow-orange.

In addition to the original host, rainbow perch (*Eupomotis gibbosus*) were tested and found to be susceptible. Seven perch were inoculated intraperitoneally or via the dorsal lymphatic sac with 0.1 ml of an emulsion of infected material. Six of these test fish died within 3 to 4 months.

The organism was an acid-fast, gram-positive, straight to slightly curved, granulated, nonmotile, nonsporulated rod.

The following authors recorded the organism's presence in the indicated hosts. Nigrelli (1943, 1953) recorded it in the neon tetra (*Hypessobrycon inessi*) and in other fish of the New York City Zoo. The German worker Reichenbach-Klinke (1955) reported the organism from several fish including the garfish (*Belone belone*), the picarel (*Spicara alcedo*), and the white or sea perch (*Morone labrax*). Vogel (1958) made a comprehensive study of the acid-fast bacillus disease in cold-blooded vertebrates. He stated that "the disease is found in 10 orders, 34 families, 84 genera, and 120 species of marine and freshwater teleosts from all over the world."

Earp *et al.* (1953) reported that acid-fast organisms were present in 50 adult fall Chinook salmon (*Oncorhynchus tschawytscha*) taken at Bonneville Dam in 1952. The following is a detailed description of the disease (Wood and Ordal, 1958)[2] in Chinook salmon.

In general, there may or may not be any external symptoms. If such symptoms do exist, they may be characterized by stunted appearance, brighter color and/or lack of secondary sexual development.

Internally the disease may present the following appearance in the organs:

Kidney: Gross lesions are seldom observed in this organ due to their small size and color which tend to blend them with the kidney tissue. The posterior end of the kidney may be soft and mushy due to a heavy infection.

Liver: There are often discrete, gray-white lesions of varying sizes scattered throughout the organ. Most often the disease is manifested as the miliary type and in some fish the entire organ appears to be replaced with acid-fast bacilli.

Spleen: Usually not enlarged. Acid-fast bacilli may be found.

Intestine: Pockets of the organism in and along the intestine and around the caeca. The bacilli

[2] The author is indebted to Mr. B. J. Earp, Washington State Department of Fisheries, and Mr. A. J. Ross, Western Fish Disease Laboratory, for contributions to the description.

may form connective membranes in the peritoneal cavity.

Genitalia: Occasionally poorly developed to the point wherein the observer is unable to determine sex. Johnstone (1927) made the same observation in his paper. Pockets of the bacilli may be found near the roe.

Most often there is no indication of the presence of the disease in a fish until tissue smears are examined. The liver is the organ of choice for smears and the Ziehl-Neelsen staining technique is recommended.

Wood and Ordal (1958) indicated that the following salmonoid fish were found to be susceptible to the acid-fast bacillus disease: the Chinook salmon (*Oncorhynchus tschawtyscha*), the silver salmon (*O. kisutch*), the blueback salmon (*O. nerka*), and both the sea-run steelhead and the resident rainbow trout (*Salmo gairdneri*), but only in hatchery stock. They were unable to find a positive case of the disease in the known wild stocks that they observed. Their findings indicate that the hatchery fish are infected by eating infected viscera and carcasses that are incorporated into their diet and that the longer the fish are held at the hatchery, the greater is the infection. Another interesting fact that these data point out is the inability of the infected fish to survive the holding period prior to spawning. This is a strong condemnation of the fish culturist's practice of feeding fry with adult salmon products.

The loss of fish, due to death from the disease; the debilitation of the fish, making them easy prey for predators, fishermen, and accident; and the sexual underdevelopment, rendering them unfit for spawning, all tend to emphasize the importance of this disease as a contributing factor to the salmon fisheries' dilemma.

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